Remarks

The specification is amended to add a paragraph reciting related applications and to conform with the requirements for patent applications containing nucleotide sequence and/or amino acid sequence disclosures. No new matter is introduced. No amendments are made to the claims. No amendments are made to the figures.

The specification is amended to add a paragraph reciting related applications. This information was included with the original transmittal letter that accompanied the request to begin national procedures under 35 U.S.C. 371(f), filed on July 23, 1999.

The specification is also amended to conform with the requirements for patent applications containing nucleotide sequence and/or amino acid sequence disclosures, as specified in the Office Action dated July 31, 2001. Applicants thank the Examiner for the opportunity to discuss with her the requirements and the manner of amending the specification to satisfy the requirements. Specifically, SEQ ID NO: identifiers are associated with particular sequences disclosed in the specification. In addition, minor typographical corrections are made to correct obvious misspellings and errors in capitalization and punctuation.

A substitute Sequence Listing is also submitted herewith, incorporating the amino acid sequence SIINFEKL as SEQ ID NO: 3. This amino acid sequence, which appears at page 16, line 14 and at page 24, line 10 of the specification, was not previously assigned a SEQ ID NO or listed in the Sequence Listing. The substitute Sequence Listing incorporates this amino acid sequence by substituting it for a duplicated nucleotide sequence originally listed as SEQ ID NO: 3, where the latter nucleotide sequence was identical to the nucleotide sequence listed as SEQ ID NO: 4. The effect of this substitution is two-fold: it removes an inadvertent duplication in the sequence listing, and it incorporates, as required under 37 C.F.R. 1.821, the amino acid sequence disclosed in the specification. The number of sequences in the substitute Sequence Listing is the same as in the previous Sequence Listing.

It is believed that the application as amended is now in conformity with the requirements for patent applications containing nucleotide sequence and/or amino acid sequence disclosures, and that the claims are in condition for allowance. Favorable action is earnestly solicited. If for any reason the examiner has any question or would require further information, she is encouraged to contact the Applicant's representative at the number presented below.

Respectfully submitted,

Alan W. Steele
Registration No. 45,128
Wolf, Greenfield and Sacks, P.C.
600 Atlantic Ave.
Boston, MA 02210
617/720-3500
Fax/617/720-2441

C1041/7005 (AWS)
October 17, 2001
X 08/31/2001
XX 01/31/2002

Attorney Docket No. C1041/7005 (AWS)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Wagner et al.
Serial No:	09/355,254
Filed:	February 22, 2000
For:	PHARMACEUTICAL COMPOSITIONS COMPRISING A POLYNUCLEOTIDE AND OPTIONALLY AN ANTIGEN ESPECIALLY FOR VACCINATION
Examiner:	J. Zara
Art Unit:	1635

CERTIFICATE OF MAILING UNDER 37 C.F.R. §1.8(a)

The undersigned hereby certifies that this document is being placed in the United States mail with first-class postage attached, addressed to Commissioner for Patents, Washington, D.C. 20231, on the 17th day of October, 2001.

Alan W. Steele, Reg. No. 45,128

Commissioner for Patents
Washington, D.C. 20231

Appendix to Response to Office Action

The following are marked up versions of paragraphs amended in the Response to the Office Action dated July 31, 2001, submitted herewith.

Page 16, second paragraph:

The pharmaceutical composition of the invention may advantageously be tested in mice. In such experiments mice were usually immunized by way of the hind footpad with 50 µl per foot of peptide liposome preparation. After four days, the draining popliteal lymph nodes (LN) were removed and a single-cell suspension was prepared. The cells were cultured for four days in the presence of IL-2 and a chromium release assay was performed utilizing the syngenic target cell EL-4 or the cell line EG-7 which is transfected with the gene for ovalbumin and thus presents ovalbumin peptides as antigen (Fig. 1). In some experiments EL-4 pulsed with the MHC class I (K^b[b]) restricted ovalbumin peptide SIINFEKL (SEQ ID NO: 3) was used as the target for kill.

Art Unit: 1635

Page 17, second paragraph:

An oligonucleotide that has been identified to be beneficial in accordance with the invention is IL-12p40 AGCTATGACGTTCCAAGG (SEQ ID NO: 10).

Page 19, second paragraph under EXAMPLE 1:

Three sequences containing the sequence motif of 5'Pu-Pu-CpG-Py-Py-3' are described in the literature for having immunostimulating properties. One sequence is derived from the ampicillin resistance gene of E. coli, here termed AMP (TCATTGGAAAACGTTCTTCGGGGC; SEQ ID NO: 1). The second sequence is derived from a BCG gene and is termed BCG-A4A (ACCGATGACGTCGCCGGTGACGGCACCACG; SEQ ID NO: 2). The third is a synthetic sequence claimed to be a prototype of bacterial CpG sequences, referred to by Krieg et[.] al. as 1668 (TCCATGACGTTCCTGATGCT; SEQ ID NO: 4). These sequences were synthesized to include a phosphorothioate linkage to reduce destruction by DNase. These oligomers served as an adjuvant in combination with ovalbumin to induce a cytolytic T cell response.

Beginning at the top of Page 21:

EXAMPLE 3

Use of eukaryotic transcription regulatory elements or sequence manipulation prevents toxic shock symptoms.

Due to toxicity, the need is established for the discovery of non-toxic sequences for safe human and animal use. Since toxicity is at issue when developing vaccine adjuvants and therapeutics, we were interested to develop oligomers that circumvented toxicity but retained immunostimulatory properties. We screened eukaryotic sequences displaying the absence of lethality but maintaining immunostimulatory qualities. One

Art Unit: 1635

such sequence was the cyclic AMP response element (CRE) which is the consensus binding site for the transcription factors CREB/ATF as well as the AP-1 family, sequence (GATTGCCTGACGTCAGAGAG; SEQ ID NO: 8) [Roesler, W. J. et al., J. Biol. Chem. 263, 9063-9066 (1988)]. Table 2 demonstrates the loss of lethality of the CRE sequence. To further evaluate the sequence specificity of these effects we made sequence exchanges between CRE and 1668. An exchange of only two nucleotides between CRE and 1668 resulted in a loss of lethality (Table 2).

Table 2 Sequences of oligomers and death due to lethal shock

a		
1668	TCCAT**GACGTT**CCTGATGCT	(SEQ ID NO: 4)
CRE	ATTGCCT**GACGTCA**GAGAGC	(SEQ ID NO: 5)
1668-CA	TCCAT**GACGTCA**CTGATGCT	(SEQ ID NO: 6)
CRE-TC	*ATTGCCT***GACGTT***C*GAGAGC	(SEQ ID NO: 7)
b		
1668	5/5	
CRE	0/5	
1668-CA	0/3	
CRE-TC	3/3	

Lethality was determin[in]ed as in [e]Example 2. The 1668 sequence fortuitously contains a combination of transcription response elements, namely the transcription factor binding sites (TGACGTTCC). This element represents the binding site for HSVIP04 (ATF), HSINS04 (CREB half site), CAMV35SR03 (HBP-1a yeast) or ADE422 (AP-1) in combination with an HSIL606 site which is a repressor site (sequence analysis from EMBL database Heidelberg). This sequence can be found in the 5' non-coding regions (promoters) of several eukaryotic cytokine genes including human IL-13 promoter and [Il]IL-12 p40 intron 1. The CRE sequence contains all the response elements cited above except for HSIL606 and it contains the full CRE pa[l]lindromic

sequence (TGACGTCA). In accordance[s] with the invention, the CRE sequence did not induce death and changes in the 1668 eliminate toxicity.

TNF-α release is a hallmark of lethal toxic shock [Tracey, K. J. et al., Science 234, 470-474 (1986), Tracey, K. J. et al., Nature 330, 662-664 (1987)]. An exchange of only two nucleotides between CRE and 1668 resulted in a loss of macrophage induced TNF-α release activity. The sequence of the corresponding oligonucleotide is given in Table 2. The reported 6-mer active core sequence of 1668 contains the CpG flanked by two 5' purines and two 3' pyrimidines. The exchange of CA for TC does not affect this motif, however, TNF-α release was severely diminished. Thus, the broader core 8-mer sequence or the transcription response element and not the surrounding sequence environment was responsible for these effects. In accordance with the invention, when utilizing macrophage derived TNF-α release as a marker, the information comprised in the prior art 5'Pu-Pu-CpG-Py-Py-3' motif alone was not satisfactory for predicting oligomer activity or toxicity. Additionally, in contrast to 1668, CRE did not induce IL-6 release in vivo or from the ANA-1 cell line in vitro.

Beginning near the bottom of Page 23:

EXAMPLE 5

ssDNA containing transcription response elements serve as adjuvant for cellular immunity.

We have described the use of liposomes in combination with Quil A or QS-21 to induce cytolytic T cells (CTL) to either soluble antigen or peptides [Lipford, G. B., Wagner, H. & Heeg, K., Vaccine 12, 73-80 (1994), Lipford, G. B. et al., J. Immunol. 150, 1212-1222 (1993)]. Liposome entrapped antigen alone was an ineffective inducer of CTL activity, but with the addition of immunostimulatory saponins the inoculum became effective. To test the in vivo T cell immunomodulatory potential of oligomers we utilized this vehicle to

Art Unit: 1635

demonstrate primary activation of CTL. Figure 1 shows a substantial primary CTL response induced by an inoculum of ovalbumin liposomes plus ssDNA matching transcription response elements. The lytic units value interpolated from these curves was approximately 500 L.U. as compared to <20 L.U. for ovalbumin liposomes only (Table 3). CTL memory, an important quality for vaccine protection, could also be demonstrated with these inocula. If mice were rested for two weeks after the first injection and reinjected with the same inoculum, CRE recalled CTL displaying lytic units measured at approximately 1500 L.U. (Table 3). Additional, when the inoculum was formulated with the immunodominant K^b restricted ovalbumin peptide SIINFEKL (SEQ ID NO: 3), the oligomers induced a specific primary CTL response. Thus, oligomers serve as a strong in vivo stimulus resulting in T cell activation and the proliferation of antigen specific CTL effectors. The inoculum can contain protein or peptide as the target antigen.

Table 3 Cytolytic T cell response induced by oligomer in [
]lytic units

	CRE	PBS
Primary CTL	526 L.U.	<20 L.U.
Secondary CTL	1555 L.U.	<20 L.U.

Several other sequences have been determined to have immunomodulatory effects. Table 4 lists tested eukaryotic transcription response elements (TRE), which are preferably used in the present invention.

Art Unit: 1635

Table 4 Sequences of eukaryotic TRE tested

CRE	GATTGCCTGACGTCAGAGAG	(SEQ ID NO: 8)
IL-13	GGAATGACGTTCCCTGTG	(SEQ ID NO: 9)
AP-1	GCTTGATGACTCAGCCGGAA	(SEQ ID NO: 11)
SP1	TCGATCGGGGCGGGGCGAGC	(SEQ ID NO: 12)
C/EBP	TGCAGATTGCGCAATCTGCA	(SEQ ID NO: 13)
E[G]RG	AGCGGGGGCGAGCGGGGGCG	(SEQ ID NO: 14)
GAS/ISRE	TACTTTCAGTTTCATATTACTCTA	(SEQ ID NO: 15)
SIE	GTCCATTTCCCGTAAATCTT	(SEQ ID NO: 16)
STAT1	TATGCATATTCCTGTAAGTG	(SEQ ID NO: 17)
STAT3	GATCCTTCTGGGAATTCCTA	(SEQ ID NO: 18)
STAT4	CTGATTTCCCCGAAATGATG	(SEQ ID NO: 19)
STAT5	AGATTTCTAGGAATTCAATC	(SEQ ID NO: 20)
STAT5/6	GTATTTCCCAGAAAAGGAAC	(SEQ ID NO: 21)
IRF-1	AAGCGAAAATGAAATTGACT	(SEQ ID NO: 22)
c-Myb	CAGGCATAACGGTTCCGTAG	(SEQ ID NO: 23)
NFkB	ATATAGGGGAAATTTCCAGC	(SEQ ID NO: 24)
HSINF	CAAAAAAATTTCCAGTCCTT	(SEQ ID NO: 25)
HSIL-6	ATGTTTTCCTGCGTTGCCAG	(SEQ ID NO: 26)
CRENFkB	CTCTGACGTCAGGGGAAATTTCCAGC	(SEQ ID NO: 27)

The relative strength of the various transcription response elements for adjuvant potential for CTL induction can be seen in [f]Fig. 2.

Art Unit: 1635

Respectfully submitted,

Alan W. Steele
Registration No. 45,128
Wolf, Greenfield and Sacks, P.C.
600 Atlantic Ave.
Boston, MA 02210
617/720-3500
Fax/617/720-2441

C1041/7005 (AWS)
October 17, 2001
X 08/31/2001
XX 01/31/2002